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(57) Abstract

Derivatives of 8-substituted xanthines which are used in the prophylaxis or therapy of fungal and yeast infections.

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" TNF INHIBITORS"

10 FIELD OF INVENTION

The present invention relates to compounds which are inhibitors of the in vivo production of Tumor Necrosis Factor (TNF), a serum protein.

BACKGROUND OF THE INVENTION

Excessive or unregulated TNF production is implicated in mediating or 15 exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions; sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, allograft rejections, 20 fever and myalgias due to infection, such as influenza, cachexia secondary to infection or malignancy, cachexia, secondary to acquired immune deficiency syndrome (AIDS), AIDS, ARC (AIDS related complex), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, or pyresis.

AIDS results from the infection of T lymphocytes with Human 25 Immunodeficiency Virus (HIV). At least three types or strains of HIV have been identified, i.e., HIV-1, HIV-2 and HIV-3. As a consequence of HIV infection, T-cell mediated immunity is impaired and infected individuals manifest severe opportunistic infections and/or unusual neoplasms. HIV entry into the T lymphocyte requires T lymphocyte activation. Other viruses, such as HIV-1, HIV-2 infect T lymphocytes after T Cell activation and such 30 virus protein expression and/or replication is mediated or maintained by such T cell activation. Once an activated T lymphocyte is infected with HIV, the T lymphocyte must continue to be maintained in an activated state to permit HIV gene expression and/or HIV replication. Monokines, specifically TNF, are implicated in activated T-cell mediated HIV protein expression and/or virus replication by playing a role in maintaining T lymphocyte 35 activation. Therefore, interference with monokine activity such as by inhibition of monokine production, notably TNF, in an HIV-infected individual aids in limiting the maintenance of T cell activation, thereby reducing the progression of HIV infectivity to previously uninfected cells which results in a slowing or elimination of the progression of immune dysfunction

caused by HIV infection. Monocytes, macrophages, and related cells, such as kupffer and glial cells, have also been implicated in maintenance of the HIV infection. These cells, like T-cells, are targets for viral replication and the level of viral replication is dependent upon the activation state of the cells. [See Rosenberg et al., The Immunopathogenesis of HIV Infection, Advances in Immunology, Vol. 57, (1989)]. Monokines, such as TNF, have been shown to activate HIV replication in monocytes and/or macrophages [See Poli, et al., Proc. Natl. Acad. Sci., 87:782-784 (1990)], therefore, inhibition of monokine production or activity aids in limiting HIV progression as stated above for T-cells. Additional studies have identified TNF-α as a common factor in the activation of HIV in vitro and has provided a clear mechanism of action via the nuclear factor κB, a nuclear regulatory protein found in the cytoplasm of cells (Osborn, et al., PNAS (86) 2336-2340). This evidence suggests that a reduction of TNF synthesis may have an antiviral effect in HIV infections, by reducing the transcription and thus virus production.

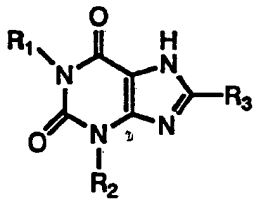
TNF has also been implicated in various roles with other viral infections, such as the cytomegalia virus (CMV), influenza virus, adenovirus, and the herpes family of viruses for similar reasons as those noted.

The ability to control the adverse affects of TNF is furthered by the use of the compounds which inhibit TNF in mammals who are in need of such use. There remains a need for compounds which are useful in treating TNF mediated disease states which are exacerbated or caused by the excessive and/or unregulated production of TNF.

Summary of the Invention

This invention relates to the use of the compounds of Formula (I) in inhibiting the production of TNF in a mammal, including humans, in need of such treatment, which method comprises administering to such mammal, an effective TNF inhibiting amount of a compound of Formula (I). More specifically the inhibition of the production of TNF is useful in the treatment, prophylactically or therapeutically, of any disease state in a mammal which is exacerbated or caused by excessive or unregulated TNF production.

The compounds of the present invention of Formula (I) are represented by the structure.



(I)

wherein

R_1 and R_2 are each independently alkyl or a moiety of the formula $-(CH_2)_m-A$; provided that at least one of R_1 and R_2 is $-(CH_2)_m-A$;

m is a number from 0 to 3;

A is an unsubstituted or substituted cyclic hydrocarbon radical;

R_3 is halogen, nitro, or $-NR_4R_5$;

R_4 and R_5 are independently hydrogen, alkyl, alkylcarbonyl or together with the nitrogen to which they are attached forming an optionally substituted heterocyclic ring; and the pharmaceutically acceptable salts thereof.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of Formula (I) are also useful in the treatment of viral infections, where such viruses are sensitive to upregulation by TNF or will elicit TNF production in vivo. The viruses contemplated for treatment herein are those that produce TNF as a result of infection, or those which are sensitive to inhibition, such as by decreased replication, directly or indirectly, by the TNF inhibitors of Formula (1). Such viruses include, but are not limited to; HIV-1, HIV-2 and HIV-3, Cytomegalovirus (CMV), Influenza, adenovirus and the Herpes group of viruses, such as but not limited to, Herpes Zoster and Herpes Simplex.

This invention more specifically relates to a method of treating a mammal, afflicted with a human immunodeficiency virus (HIV), which comprises administering to such mammal an effective TNF inhibiting amount of a compound of Formula (I).

The compounds of Formula (I) are also useful in the treatment of yeast and fungal infections, where such yeast and fungi are sensitive to upregulation by TNF or will elicit TNF production in vivo. A preferred disease state for treatment is fungal meningitis. Additionally, the compounds of Formula (I) may be administered in conjunction with other drugs of choice for systemic yeast and fungal infections. Drugs of choice for fungal infections include but are not limited to colistin, nystatin, the class of compounds called the polymi such as Polymycin B, the class of compounds called the imidazoles, such as clotrim e, econazole, miconazole, and ketoconazole; the class of compounds called the triazoles, such as fluconazole, and the class of compound called the Amphotericins, in particular Amphotericin B. Additional antifungal agents include antimycin A; azaserine; flucytosine; hexetidine; nifuratel; marcolides compounds such as candicidin, griseofulvin, and tolnaftate.

The co-administration of the anti-fungal agent with a compound of Formula (I) may be in any preferred composition for that compound such as is well known to those skilled in the art, for instance the various Amphotericin B formulations. Co-administration of an anti-fungal agent with a compound of Formula (I) may mean simultaneous administration or in practice, separate administration of the agents to the mammal but in a

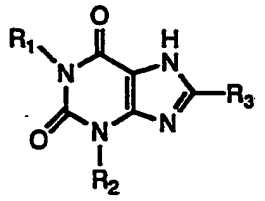
consecutive manner. In particular, the compounds of Formula (I) may be co-administered with a formulation of Amphotericin B, notably for systemic fungal infections. The preferred organism for treatment is the Candida organism. The compounds of Formula (I) may be co-administered in a similar manner with anti-viral or anti-bacterial agents.

The compounds of Formula (I) may also be used for inhibiting &/or reducing the toxicity of an anti-fungal, anti-bacterial or anti-viral agent by administering an effective amount of a compound of Formula (I) to a mammal in need of such treatment. Preferably, a compound of Formula (I) is administered for inhibiting or reducing the toxicity of the Amphotericin class of compounds, in particular Amphotericin B.

The compounds of Formula (I) may also be used in association with the veterinary treatment of mammals, other than in humans, in need of inhibition of TNF production. TNF mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted above, but in particular viral infections. Examples of such viruses include, but are not limited to, feline immunodeficiency virus (FIV) or other retroviral infection such as equine infectious anaemia virus, caprine arthritis virus, visna virus, maedi virus and other lentiviruses.

A preferred method of this invention is the treatment, therapeutically or prophylactically, of viral infections, in particular where such viruses are sensitive to upregulation by TNF or will elicit TNF production in vivo by administering an effective amount of a compound of Formula (I) or most preferably, the compound 1,3-di-cyclopropylmethyl-8-amino xanthine or a pharmaceutically acceptable salt thereof.

The compounds of the present invention of Formula (I) are represented by the structure:



(I)

wherein

R_1 and R_2 are each independently alkyl or a moiety of the formula - $(CH_2)_m$-A;

m is a number from 0 to 3;

A is an unsubstituted or substituted cyclic hydrocarbon radical;

R_3 is halogen, nitro, or $-NR_4R_5$;

R_4 and R_5 are independently hydrogen, alkyl, alkylcarbonyl or together with the nitrogen to which they are attached forming an optionally substituted heterocyclic ring; and the pharmaceutically acceptable salts thereof.

Preferably both R_1 and R_2 represent $-(CH_2)_m$-A. Preferably the A moiety represents a C_{3-8} cycloalkyl group, particularly a C_{3-6} cycloalkyl and preferably

unsubstituted. More preferably A is a cyclopropyl or cyclobutyl moiety. Preferably m is zero or one. Suitable optional substituent groups for any cyclic hydrocarbon include a C_{1-6}alkyl moiety or halogen atom.

A preferred group for R_1 or R_2 is an alkyl group of 1 to 6 carbons, specifically methyl, ethyl, propyl or n-butyl. More preferred is n-butyl.

When R_3 is halogen, the preferred substitution is bromine or chlorine.

When R_3 is $-NR_4R_5$, and R_4 and R_5 represent alkyl or alkylcarbonyl, it is preferred that one of R_4 or R_5 is hydrogen.

Suitable heterocyclic groups include saturated or unsaturated heterocylic groups having single or fused rings, each ring having 5 to 7 ring atoms which ring atoms optionally comprise up to two additional hetero atoms selected from O, N, or S.

Preferred heterocyclic groups include single rings comprising 5 to 7 ring atoms, more preferably 5 to 6 ring atoms,and most preferably 6 ring atoms. Preferred heterocyclic groups are pyrrolidinyl, piperidinyl, or morpholinyl rings.

Specifically exemplified compounds of Formula (I) are:

1,3-di-n-butyl-8-nitro xanthine;
1,3-di-cyclopropylmethyl-8-nitro xanthine;
1,3-di-cyclobutylmethyl-8-nitro xanthine;
1,3-di-cyclopentylmethyl-8-nitro xanthine;
1,3-di-cyclohexylmethyl-8-nitro xanthine;
1,3-di-n-butyl-8-amino xanthine;
1,3-di-cyclopropylmethyl-8-amino xanthine;
1,3-di-cyclobutylmethyl-8-amino xanthine;
1,3-di-cyclopentylmethyl-8-amino xanthine;
1,3-di-cyclohexylmethyl-8-amino xanthine;
1,3-di-cyclopropyl-8-amino xanthine;
1,3-di-n-butyl-8-acetamido xanthine;
1,3-di-n-butyl-8-chloro xanthine;
1,3-di-n-butyl-8-bromo xanthine;
1,3-di-cyclopropylmethyl-8-chloro xanthine;
1,3-di-cyclohexyl-8-chloro xanthine;
1,3-di-n-butyl-8-piperidino xanthine;
1,3-di-cyclopropylmethyl-8-morpholino xanthine;
1,3-di-n-butyl-8-pyrrolidinyl xanthine;
1,3-di-cyclopropylmethyl-8-pyrrolidinyl xanthine;
1,3-di-cyclopropylmethyl-8-piperidinyl xanthine;
1,3-di-cyclohexylmethyl-8-piperidinyl xanthine;

1,3-di-cyclohexylmethyl-8-bromo xanthine; and

1,3-di-cyclohexyl-8-nitro xanthine; or the pharmaceutically acceptable salts thereof.

The most preferred compound of Formula (I) for use in the methods of this invention is 1,3-di-cyclopropylmethyl-8-amino xanthine or a pharmaceutically acceptable salt thereof.

By the term "alkyl" groups as used herein, alone or when used as part of another group (for example as in alkylcarbonyl) is meant to include both straight or branched chain radicals of 1 to 12 carbon atoms, unless the chain length is limited thereto, including, but not limited to methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, and the like.

By the term "cyclic hydrocarbon", unless specified otherwise, as used herein is meant a single ring or a fused rings of 3 to 8 carbon atoms. Cyclic hydrocarbons may comprise up to 8 carbons in each ring. The term "cycloalkyl" or "cycloalkyl alkyl" as used herein is meant to be interchangeable with the term "cyclic hydrocarbon". Cycloalkyl and cycloalkyl-alkyl groups are meant to include, but not limited to cyclopropyl, cyclopropyl-methyl, cyclopentyl or cyclohexyl.

By the term "halo" as used herein is meant all halogens, i.e., chloro, fluoro, bromo and iodo.

By the term "inhibiting the production of IL-1" or "inhibiting the production of TNF" is meant

a) a decrease of excessive in vivo IL-1 or TNF levels, respectively, in a mammal, specifically humans, to normal levels or below normal levels by inhibition of the in vivo release of IL-1 by all cells, including but not limited to monocytes or macrophages;

b) a down regulation, at the translational or transcription level, of excessive in vivo IL-1 or TNF levels, respectively, in a mammal, specifically humans, to normal levels or below normal levels; or

c) a down regulation, by inhibition of the direct synthesis of IL-1 or TNF levels as a postranslational event.

By the term "TNF mediated disease or disease states" is meant any and all disease states in which TNF plays a role, either by production of TNF itself, or by TNF causing another cytokine to be released, such as but not limited to IL-1, or IL-6. A disease state in which IL-1, for instance is a major component, and whose production or action, is exacerbated or secreted in response to TNF, would therefore be considered a disease state mediated by TNF. As TNF-ß (also known as lymphotoxin) has close structural homology with TNF-α (also known as cachectin) and since each induces similar biologic responses and binds to the same cellular receptor, both TNF-α and TNF-ß are inhibited by the compounds

of the present invention and thus are herein referred to collectively as "TNF" unless specifically delineated otherwise. Preferably TNF-α is inhibited.

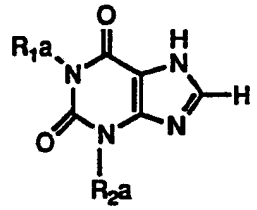
By the term "cytokine" as used herein is meant any secreted polypeptide that affects the functions of cells, and is a molecule which modulates interactions between cells in the immune or inflammatory response. A cytokine includes, but is not limited to monokines and lymphokines regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a mononuclear cell, such as a macrophage and/or monocyte but many other cells produce monokines, such as natural killer cells, fibroblasts, basophils, neutraphils, endothelial cells, brain astrocytes, bone marrow stromal cells, epideral keratinocytes, and ß- lymphocytes. Lymphokines are generally referred to as being produced by lymphoctye cells. Examples of cytokines for the present invention include, but are not limited to, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-α) and Tumor Necrosis Factor beta (TNF-ß).

The inhibition of a cytokine, contemplated by the present invention, for use in the treatment of a HIV-infected human, must be a cytokine which is implicated in (a) the initiation and/or maintenance of T cell activation and/or activated T cell-mediated HIV gene expression and/or replication, and/or (b) any cytokine-mediated disease associated problem such as cachexia or muscle degeneration. The cytokine specifically desired to be inhibited is TNF α.

METHODS OF PREPARATION

The preparation of the compounds of Formula (I) can be carried out by one of skill in the art according to the procedures outlined herein .

A process for the preparation of a compound of formula (I), which process comprises reacting a compound of formula (II):



wherein R^{1a} represents R^1, as defined in relation to formula (I), or a group convertible to R^1 and R^{2a} represents R^2, as defined in relation to formula (I), or a group convertible thereto, with a reagent capable of substituting the C-8 hydrogen of the compound of formula (II) with a group R^{3a} wherein R^{3a} represents R^3, as defined above in relation to formula (I), or a group convertible thereto; and thereafter, if required carrying out ne or more of the following optional steps:

(i) converting any group R^{1a} to R^1 and/or R^{2a} to R^2;

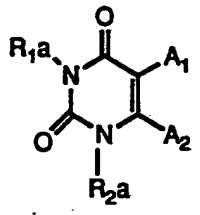
(ii) converting a compound of formula (I) into a further compound of formula (I);

(iii) converting a compound of formula (I) into a pharmaceutically acceptable salt.

Suitable reagents for substituting the C-8 hydrogen of the compound of formula (II) with a group R^{3a} are well known, conventional, reagents. The conditions of reaction for the substitution of the C-8 hydrogen of the compound of formula (II) will of course depend upon the particular reagent chosen, and in general the conditions used will be those which are conventional for the reagent used. One particularly suitable reagent is a nitrating agent.

In one convenient form of the above mentioned process the compound of formula (II) is reacted with a suitable nitrating agent to provide a compound of formula (I) wherein R^3 represents a nitro group and then converting the nitro group into a halogen atom or a group of the above defined formula $-NR^4R^5$.

A compound of formula (II) may be prepared by the dehydrating cyclization of a compound of formula (III):



(III)

wherein R^{1a} represents R^1, as defined in relation to formula (I), or a group convertible to R^1 and R^{2a} represents R^2, as defined in relation to formula (I), or a group convertible thereto, A^1 represents -NO or -NHCHO and A^2 represents $-NHCH_3$ or NH_2, providing that when A^1 is -NO then A^2 is $-NHCH_3$ and when A^1 is -NHCHO then A^2 is NH_2; and thereafter, if required, converting any group R^{1a} to R^1 and/or R^{2a} to R^2. The dehydrating cyclization of a compound of formula (III) may be carried out under any suitable conditions. Favorably the conditions chosen are these wherein the water formed is removed from the reaction mixture, thus the reaction is generally carried out at an elevated temperature in the range of from 100°C to 200°C such as in the range of 180°C to 190°C.

In one aspect of the process, especially when A^1 is -NO and A^2 is $-NHCH_3$, the reaction is carried out in a solvent immiscible with water, such as toluene, at the reflux temperature of the solvent, the water being removed using a water-separator.

Suitable values for R^{1a} and R^{2a} include R^1 and R^2 respectively or nitrogen protecting groups such as benzyl groups.

When R^{1a} or R^{2a} represents other than R^1 or R^2 respectively, the above mentioned conversions of R^{1a} into R^1 and R^{2a} to R^2 may be carried out using the appropriate conventional procedure. For example when R^{1a} (or R^{2a}) represents a nitrogen protecting group, such as a benzyl group, the protecting group may be removed using the appropriate conventional procedure, such as catalytic hydrogenation, and the resulting product reacted with a compound of formula (IV):

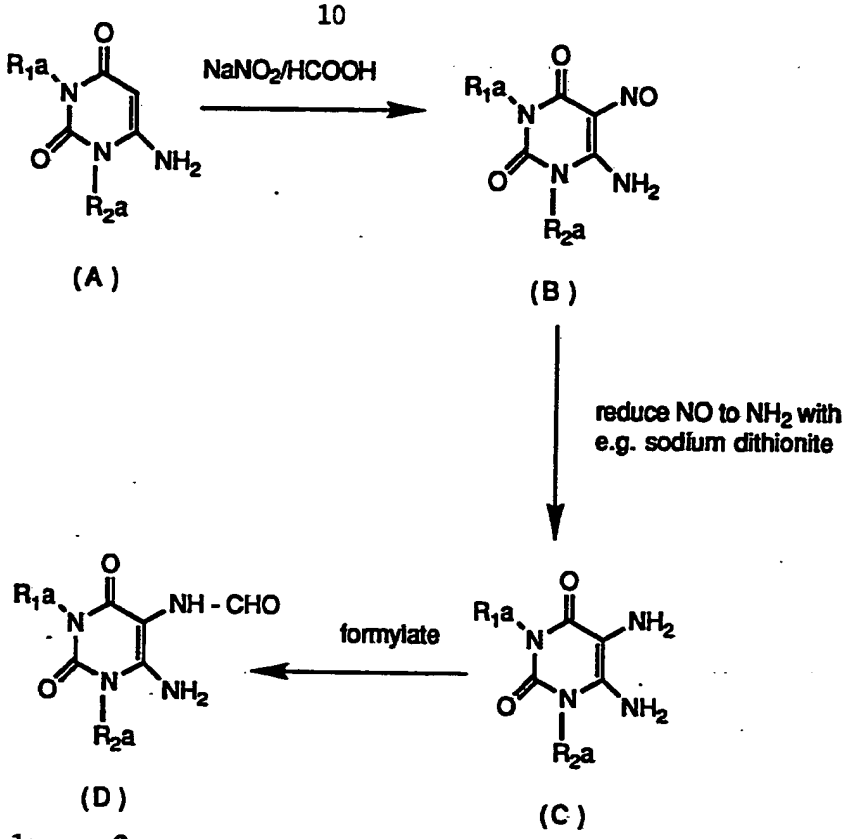
$$X\text{-}(CH_2)_m\text{-}A \quad (IV)$$

wherein A and m are as defined in relation to formula (I) and X represents a suitable leaving group, such as halide, for example bromide or iodide.

The protection of any reactive group or atom, such as the xanthine nitrogen atom may be carried out at any appropriate stage in the aforementioned process. Suitable protection groups include those used conventionally in the art for the particular group or atom being protected, for example suitable protecting groups for the xanthine nitrogen atoms are benzyl groups. Such protecting groups are known to those skilled in the art and are readily disclosed in Greene, T., Protective Groups in Organic Synthesis, Wiley Publishers, NY (1981), the contents of which are hereby incorporated by reference. Protecting groups may be prepared and removed using the appropriate, conventional procedures such as illustrated below:

For example, N-benzyl protecting groups may be prepared by treating the appropriate compound of formula (II) with benzyl chloride in the presence of a base such as triethylamine. The N-benzyl protection groups may be removed by catalytic hydrogenation over a suitable catalyst, such as palladium on activated charcoal, in a suitable solvent, such as ethanol conveniently at an elevated temperature, or by treatment with anhydrous aluminium chloride in dry benzene at ambient temperature.

A compound of formula (III) wherein A^1 represents -NHCHO and R^2 represents-NH_2 may suitably be prepared from a 6-aminouracil of formula (A) according to the following reaction scheme:

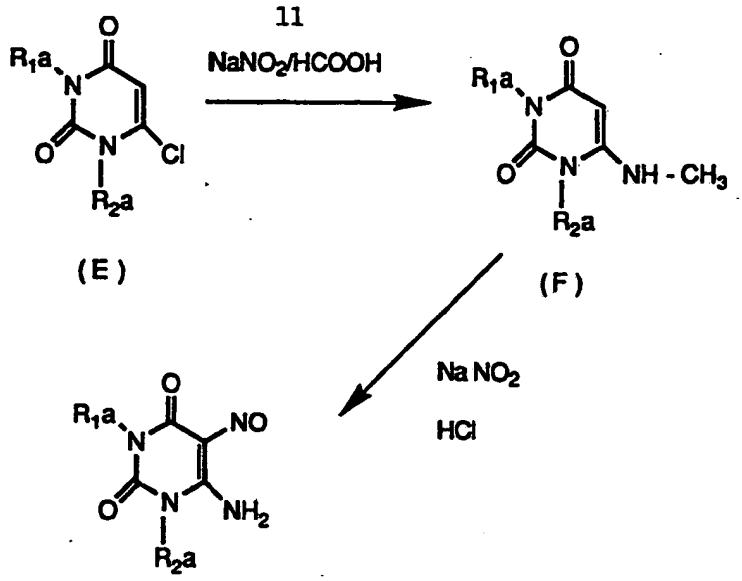


wherein R^{1a} and R^{2a} are as defined in relation to formula (II).

Suitably, the reaction conditions used in the above mentioned reaction scheme are appropriate conventional conditions. In a preferred aspect of the process, the conversion of the 6-aminouracil (A), via (B) and (C), to the corresponding compound of formula (III) and the cyclization of the compound of formula (III) to the compound of formula (II) are all carried out in-situ, suitably by using an analogous procedure to that of H. Bredereck and A. Edenhofer, Chem. Berichte, 88, 1306-1312 (1955).

The 6-aminouracils of formula (A) may themselves be prepared by the method of V. Papesch and E.F. Schroder, J. Org. Chem., 16, 1879-90 (1951), or Yozo Ohtsuka, Bull. Chem. Soc. Jap., 1973, 46(2), 506-9.

A compound of formula (III) wherein A^1 represents -NO and A^2 represents $-NHCH_3$ may conveniently be prepared from a 6-chlorouracil of formula (E), according to the following reaction scheme:



wherein R^{1a} and R^{2a} are as defined in relation to formula (II).

Suitably, the reaction conditions used in the last above mentioned scheme are the appropriate conventional conditions, for example those used in the method of H. Goldner, G. Dietz and E. Carstens, Liebigs Annalen der Chemie, 691, 142-158 (1965). The 6-chlorouracil of formula (D) may also be prepared according to the procedure of Dietz et al.

When R^3 represent a nitro group, suitable conversions of the nitro group into another group R^{3a} include the following:

(i) converting the nitro group into a halogen atom;

(ii) converting the nitro group into an amine group;

(iii) converting the nitro group into a halogen atom followed by conversion of the halogen atom into a group $-NR^4R^5$ wherein R^4 and R^5 together with the nitrogen atom to which they attached form an optionally substituted heterocyclic group; and

(iv) converting the nitro group into an amino group and thereafter alkylating and/or acylating the amino group to provide a group $-NR^4R^5$ wherein R^4 represents hydrogen, alkyl or alkylcarbonyl and R^5 represents alkyl or alkylcarbonyl.

A nitro group may be converted into a halogen atom by using any convenient halogenating agent. One suitable halogenating agent is a hydrogen halide, suitably reacted in aqueous conditions for example by using concentration hydrochloric acid or concentrated

hydrobromic acid at an elevated temperature, for example in the range of from 50 to 150°C.

A further suitable halogenating agent is a phosphorous oxyhalide, such as phosphorous oxychloride, which may be reacted in any suitable solvent, such as dimethylformamide,suitably at an elevated temperature for example in the range of from 50°C to 150°C.

A nitro group may conveniently be converted into an amino group by conventional reduction methods for example by using tin powder and concentrated hydrochloric acid at ambient temperature or by using sodium dithionite in aqueous methanol at ambient temperature.

When R^3 in the compound of formula (I) represents a halogen atom it may be converted into a group $-NR^4R^5$ by reacting with a reagent of formula (III):

$$-HNR^{4a}R^{5a} \quad \text{(III)}$$

wherein R^{4a} and R^{5a} are as defined above as R_4 and R_5 in Formula (I) respectively.

The reaction between the compound of formula (I) and the compound of formula (III) may be carried out in any suitable solvent, such as toluene, at any temperature providing a convenient rate of formation of the product, but suitably at an elevated temperature, such as in the range of from 50° to 180°C, at atmospheric or an elevated pressure.

Suitable alkylation methods for use in the above mentioned conversions include those used conventionally in the art, for example methods using halides, preferably iodides, in the presence of a base such as potassium carbonate in any convenient solvent for example acetonitrile or toluene.

Suitable acylation methods for use in the above mentioned conversions include those used conventionally in the art, thus an amino group may be converted into an alkylcarbonyl amino group by using an appropriate acylating agent, for example an amino group may be converted to an acetylamino group by using acetic anhydride at elevated temperature.

METHODS OF TREATMENT

The compounds of Formula (I) or a pharmaceutically acceptable salt thereof can also be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human, or other mammal, which is exacerbated or caused by excessive or or unregulated TNF production by such human's cell, such as but not limited to monocytes and/or macrophages, especially caused by excessive or unregulated TNF production. The compounds of Formula (I) are administered in an amount sufficient to inhibit TNF production such that it is regulated down to normal levels, or in some case to

subnormal levels, so as to ameliorate or prevent the disease state. Abnormal levels of TNF, for the present invention, constitute levels of 1) free (not cell bound) TNF, greater than or equal to 1 picogram per ml; 2) any cell associated TNF; or 3) the presence of TNF mRNA above basal levels in cells or tissues in which TNF is produced.

There are many disease states in which excessive or unregulated TNF production by monocytes and/or macrophages is implicated in exacerbating and/or causing the disease. These include endotoxemia and/or toxic shock syndrome [See Tracey et al., Nature 330:662-664 (1987); and Hinshaw et al., Circ. Shock 30:279-292 (1990)]; cachexia [See, Dezube et al., Lancet, 335 (8690):662 (1990)]; Adult Respiratory Distress Syndrome where TNF concentration in excess of 12,000 pg/ml have been detected in pulmonary aspirates from ARDS patients. [See, Millar et al., Lancet 2(8665):712-714 (1989). Systemic infusion of recombinant TNF resulted in changes typically seen in ARDS [See; Ferrai-Baliviera et al., Arch. Surg. 124(12):1400-1405 (1989)]; AIDS viral replication of latent HIV in T-cell and macrophage lines can be induced by TNF [See, Folks et al., PNAS 86:2365-2368 (1989)]. A molecular mechanism for the virus inducing activity is suggested by TNF's ability to activate a gene regulatory protein (NF-kB) found in the cytoplasm of cells, which promotes HIV replication through binding to a viral regulatory gene sequence (LTR) [See, Osborn et al., PNAS 86:2336-2340 (1989)]. TNF in AIDS associated cachexia is suggested by elevated serum TNF and high levels of spontaneous TNF production in peripheral blood monocytes from patients [See, Wright et al., J. Immunol. 141(1):99-104 (1988)].

TNF in Bone Resorption Diseases, including arthritis, wherein it has been determined that when activated, leukocytes will produce a bone-reasorbing activity, and data suggests that TNF-α and TNF-ß both contribute to this activity. [See e.g., Bertolini et al., Nature 319:516-518 (1986) and Johnson et al., Endocrinology 124(3):1424-1427(1989)]. It has been determined that TNF stimulates bone resorption and inhibits bone formation in vitro and in vivo through stimulation of osteoclast formation and activation combined with inhibition of osteoblast function. Although TNF may be involved in many bone resorption diseases, including arthritis, the most compelling link with disease is the association between production of TNF by tumor or host tissues and malignancy associated hypercalcemia [See, Calci. Tissue Int. (US) 46(Suppl.):S3-10 (1990)]. In Graft versus Host Reaction, increased serum TNF levels have been associated with major complication following acute allogenic bone marrow transplants [See, Holler et al., Blood, 75(4):1011-1016(1990)].

Cerebral malaria, which is a lethal hyperacute neurological syndrome associated with high blood levels of TNF and is the most severe complication occuring in malaria patients. A form of experimental cerebral malaria (ECM) that reproduces some

features of the human disease was prevented in mice by administration of an anti-TNF antibody. [See, Grau et al., Imm. Review 112:49-70 (1989)]. Levels of serum TNF correlated directly with the severity of disease and prognosis in patients with acute malaria attacks [See Grau et al., N. Engl. J. Med. 320(24):1586-1591 (1989)].

Another disease state in which TNF plays a role is the area of chronic Pulmonary Inflammatory Diseases. The deposition of silica particles leads to silicosis, a disease of progressive respiratory failure caused by a fibrotic reaction. Antibody to TNF completely blocked the silica-induced lung fibrosis in mice [See Piguet et al., Nature, 344:245-247 (1990)]. High levels of TNF production (in the serum and in isolated macrophages) have been demonstrated in animal models of silica and asbestos induced fibrosis [See Bissonnette et al., Inflammation 13(3):329-339 (1989)]. Alveolar macrophages from pulmonary sarcoidosis patients have also been found to spontaneously release massive quantities of TNF as compared with macrophages from normal donors [See Baughman et al., J. Lab. Clin. Med. 115(1):36-42 (1990)].

TNF is also implicated in another acute disease state such as the inflammatory response which follows reperfusion, called Reperfusion Injury and is a major cause of tissue damage after loss of blood flow [See, Vedder et al., PNAS 87:2643-2646 (1990)]. TNF also alters the properties of endothelial cells and has various pro-coagulant activities, such as producing an increase in tissue factor pro-coagulant activity and suppression of the anticoagulant protein C pathway as well as down-regulating the expression of thrombomodulin [See, Sherry et al., J.Cell Biol. 107:11269-1277 (1988)].

TNF also has pro-inflammatory activities which together with its early production (during the initial stage of an inflammatory event) make it a likely mediator of tissue injury in several important disorders including but not limited to, myocardial infarction, stroke and circulatory shock. Of specific importance may be TNF-induced expression of adhesion molecules, such as intercellular adhesion molecule (ICAM) or endothelial leukocyte adhesion molecule (ELAM) on endothelial cells [See, Munro et al., Am. J. Path. 135(1):121-132 (1989)].

TNF is also associated with yeast and fungal infections. Specifically Candida Albicans has been shown to induce TNF production in vitro in human monocytes and natural killer cells. [See Riipi et al., Infection and Immunity, Vol. 58, No.9, p. 2750-54 (1990); and Jafari et al., Journal of Infectious Diseases, Vol. 164, p. 389-95 (1991). See also Wasan et al., Antimicrobial Agents and Chemotherapy, Vol. 35, No. 10, p. 2046-48 (1991) and Luke et al., Journal of Infectious Diseases, Vol. 162, p. 211-214 (1990)].

The compounds of Formula (I) may also be used topically in the treatment or prophylaxis of topical disease states mediated or exacerbated by excessive TNF

production, respectively, such as viral infections, such as those caused by the herpes viruses, or viral conjunctivitis, etc.

In short, the treatment of TNF mediated disease includes but is not limited to such diseases as rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions; sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoisosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, acute graft rejection, allograft rejections, fever and myalgias due to infection, such as influenza, cachexia secondary to infection or malignancy, cachexia, secondary to acquired immune deficiency syndrome (AIDS), AIDS, ARC (AIDS related complex), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, pyresis, and viral infections.

All the compounds of Formula (I) are useful in the method of the subject invention, i.e. methods of inhibiting the production, preferably by macrophages, monocytes or macrophages and monocytes in a mammal, specifically a human, in need of such treatment. The method of the invention is preferably used to treat, prophylactically or therapeutically, TNF mediated disease states which are not mediated by the phospho-diesterase (PDEIV) enzyme. Preferably, the method of this invention is used in a disorder other than one associated with increased numbers of eosinophils, such as proliferative skin disease states, i.e. psoriasis, atopic dermatitis, non-specific dermititis, primary irritant contact dermatitis, allergic contact dermititis, or allergic disorders such as atopy, uticaria, eczema, rhinitis, serborrheic dermatitis, and mange in domestic animals, as disclosed in Maschler et al., Great Britain Patent Application No. 8906792.0 filed on March 23, 1989, whose entire disclosure is incorporated herein by reference. The compounds of Formula (I) may, however, be administered concurrently with another agents useful for the treatment of diseases associated with the inhibition or mediation of PDE IV or associated with increased number of eosinophils, with neuronal degeneration resulting from cerebral ischaemic events, such as surgery or stroke, or with those diseases associated with bronchodilator activity such as reversible airways obstruction, or asthma.

In addition, the present invention attributes many of the biological disease states attributable to interleukin-1 (IL-1) activity as being attributable to that of TNF activity as well. A comprehensive listing of IL-1 activities can be found in Dinarello, J. Clinical Immunology, 5 (5), 287-297 (1985). It should be noted that some of these effects have been described by others as indirect effects of IL-1.

Interleukin-1 (IL-1) has been demonstrated to mediate a variety of biological activities thought to be important in immunoregulation and other physiological conditions such as inflammati n [See, e.g., Dinarello et al., Rev. Infect. Disease, 6, 51 (1984)]. The

myriad f known biological activities of IL-1 include the activation of T helper cells, induction of fever, stimulation of prostaglandin or collagenase production, neutrophil chemotaxis, induction of acute phase proteins and the suppression of plasma iron levels. These disease states are also considered appropriate disease states of TNF activity and hence compounds of Formula (I) are also useful in their treatment as well, and the use of the compounds of Formula (I) should not be considered solely limited to the specifically described TNF mediated disease states herein. The compounds of the present invention are, therefore, efficacious in the treatment of an IL-1 mediated disease state as TNF and IL-1 act in a synergistic manner. TNF as well mediates the release, in some instances, of the monokine IL-1, therefore a reduction in the levels of TNF may be useful in the treatment of a disease state wherein IL-1 is a major component.

The present invention also relates therefore, to an effective, TNF production inhibiting amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, useful in treating, prophylactically or therapeutically, any disease state in a human which is exacerbated or caused by excessive or unregulated IL-1 production , i.e. where IL-1 is a major component, by such human's monocytes and/or macrophages.

The method of treatment and monitoring for an HIV-infected human manifesting immune dysfunction or cytokine-mediated disease associated problems is taught in Hanna, WO 90/15534, December 27, 1990. In general, an initial treatment regimen can be copied from that known to be effective in interfering with TNF activity for other TNF mediated disease states by the compounds of Formula (1). Treated individuals will be regularly checked for T cell numbers and T4/T8 ratios and/or measures of viremia such as levels of reverse transcriptase or viral proteins, and/or for progression of monokine-mediated disease associated problems such as cachexia or muscle degeneration. If no effect is seen following the normal treatment regimen, then the amount of the monokine activity interfering agent administered is increased, e.g., by fifty percent per week.

In an HIV-infected human manifesting monokine-mediated disease associated problems such as cachexia, treatment with an effective amount of a monokine activity interfering agent will initially result in a slowing of the rate of the progression of the disease associated problem, thereby slowing disease progression. It is expected that the progression of the disease associated problem will eventually cease and reverse, thereby enhancing the quality of life of the HIV-infected individual treated in such a manner. The compounds of Formula (I) are useful in the method of treatment for all disease states associated with and HIV infection, such as immune abnormalities, immune dsyfunction AIDS Related Complex (ARC) and what is referred to as acquired immune defiency syndrome (AIDS) itself. The compounds of Formula (I) will also be useful in reducing or eliminating the inflammation related damage/pathology caused by opportunistic (secondary)

infection, such as but not limited to Pneumocystic pneumonia, or cytomeglovirus infections.

It will be recognized by one of skill in the art that the actual amount of a monokine activity interfering agent required for therapeutic effect will, of course, vary with the agent chosen, the route of administration desired, the nature and severity of the HIV-infection and the particular condition of the HIV-infected human undergoing treatment, and is ultimately at the discretion of the physician. It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a monokine activity interfering agent will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of the monokine, TNF, activity interfering agent given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The compounds of Formula (I) may be administered orally (when active by this route), topically, parenterally or by inhalation in conventional dosage forms prepared by combining such agent with standard pharmaceutical carriers according to conventional procedures in an amount sufficient to produce therapeutic TNF activity interfering activity.

The pharmaceutical carrier employed can be readily determined by one of skill in the art who will recognize that such determination will depend upon various well-known factors such as the nature, quantity and character of the particular monokine activity interfering agent being employed and the form and route of administration desired. The carriers employed may be those described elsewhere herein.

In order to use a compound of the Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

The pharmaceutical composition of the present invention will comprise an effective, non-toxic amount of a compound of Formula (I) and a pharmaceutically acceptable carrier or diluent. The compounds of Formula (I) are administered in conventional dosage forms prepared by combining a compound of Formula (I) in an amount sufficient to produce TNF production inhibiting activity, respectively, with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary f liquid carriers are syrup, peanut oil, olive oil, polyethylene glycol, coconut oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax.

Compounds of Formula (I) and their pharmaceutically acceptable salts can be employed in a wide variety of pharmaceutical forms. The preparation of a pharmaceutically acceptable salt will be determined by the nature of the compound itself, and can be prepared by conventional techniques readily available to one skilled in the art. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1 gram. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, polyethylene glycol, coconut oil, glycerine or water with a flavouring or colouring agent.

The amount of a compound of Formula (I) required for therapeutic effect on topical administration will, of course, vary with the compound chosen, the nature and severity of the inflammatory condition and the animal undergoing treatment, and is ultimately at the discretion of the physician.

The term 'parenteral' as used herein includes intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. Appropriate dosage forms for such administration may be prepared by conventional techniques.

Typical parenteral compositions consist of a solution or suspension of the compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil. The daily dosage regimen for inhibition of TNF production, via parenteral administration is suitably about 0.001 mg/Kg to 40 mg/Kg, preferably about

0.01 mg/Kg to 20 mg/Kg, of a compound of the formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

The compounds of Formula (I) may be administered orally. The daily dosage regimen for oral administration is suitably about .1 mg/kg to 1000mg day. For administration the dosage is suitably about .001mg/kg to 40mg/kg, preferably about 0.01 to 20 mg/Kg of a compound of formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit activity.

The compounds of Formula (I) may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The daily dosage regimen for inhalation administration is suitably about .001 mg/kg to 40mg/kg, preferably 0.01 to 20 mg/Kg of a compound of formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer to himself a single dose.

The compounds of Formula (I) may also be administered topically. By topical administration is meant non-systemic administration and includes the application of a compound of Formula (I) externally to the epidermis, to the buccal cavity and instillation of such a compound into the ear, eye and nose, and where the compound does not significantly enter the blood stream. Thus, the compounds of Formula (I) may be administered topically in the treatment or prophylaxis of inflammatory topical disease states mediated or exacerbated by excessive TNF production, respectively, such as eczema, psoriasis or other inflammatory skin conditions such as sunburn; inflammatory eye conditions including conjunctivitis; pyresis, pain and other conditions associated with inflammation, herpes or other topical viral infections. The daily dosage regimen for topical administration is suitably about .001 mg/kg to 100mg/kg, preferably 0.1 to 20 mg/Kg of a compound of formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration.

By topical administration is meant non-systemic administration and includes the application of a compound of Formula (I) externally to the epidermis, to the buccal

cavity and instillation of such a compound into the ear, eye and nose, and where the compound does not significantly enter the blood stream.

While it is possible for an active ingredient to be administered alone as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g. from 1% to 2% by weight of the formulation although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

The topical formulations of the present invention comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredient(s). The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a

greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactants such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient, a compound of Formula (I), with which it is to be combined, the route of administration and other well-known variables.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of Formula (I) or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of Formula (I) or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

FORMULATION EXAMPLES

Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients. Examples of liquid formulations are given below.

1. A solution containing a compound of Formula (I) is prepared by dissolving the compound in water, or other suitable carrier, with or without a preservative, such as benzoic acid, to deliver the desired amount of drug per use. The compound is present in an amount from about 10μg to about 30 μg/ per ml of carrier.

2. A solution containing a compound of Formula (I) is prepared by dissolving the compound in an amount from about 1 to about 10mg per ml of PEG 400 with or without BHA/BHT preservatives. The solution can alternatively be filled into a soft gelatin capsule to prepare a solid oral dosage form or used as a syrup.

3. A solid dosage form containing a compound of Formula (I), such as 1,3-di-cyclopropylmethyl-8-amino xanthine has been prepared by mixing 50mg of the compound with various concentration (mg) of mannitol, hydroxypropylmethylcellulose, calipharm, Starch 1500, and magnesium sterate (as a lubricant), to fill capsules of an appropriate size or

the composition may, if desired, be compressed into tablets. Various formulation of the ingredients are presented in Table 1, numbered from 1 to 6.

TABLE 1

No.	cmpd. (I) (mg)	Mannitol (mg)	Explotab* (mg)	H.P.M.C.* (mg).	Mg. Sterate (mg)	CLPVP* (mg)	Lactose (mg)	Calipharm (mg)	Starch (mg)	PEG 6000 (mg)	Dose Weight (mg)
1	50.0	78.0	8.0	12.5	1.5						150
2	50.0	78.0		12.5	1.5	8.0					150
3	50.0		8.0	12.5	1.5		78.0				150
4	50.0		8.0	12.5	1.5			78.0			250
5	50.0								180.0		230
6	50.0									300.0	350

* EXPLOTAB - sodium starch glycolate
* HPMC - hydroxypropylmethylcellulose
* CLPVP - cross linked polyvinylpyrolidone
* CALIPHARM - milled calcium di-phosphate

UTILITY EXAMPLES

Example A

Inhibitory Effect of compounds of Formula (I) on in vitro TNF production by Human Monocytes

Section I: Assay set-up

The effects of compounds of Formula (I) on the in vitro production of TNF by human monocytes was examined using the following protocol.

Human peripheral blood monocytes were isolated and purified from either blood bank buffy coats or platelet pheresis residues, according to the procedure of Colotta, R. et al., J. Immunol., 132(2):936 (1984). The monocytes were plated at a density of 1 X 10^6 cells/ml medium/well in 24-well multi-dishes. The cells were allowed to adhere for 1 hour after which time the supernatant was aspirated and 1 ml fresh medium (RPMI-1640 (Whitaker Biomedical Products, Whitaker, CA) containing 1% fetal calf serum and penicillin and streptomycin at 10 units/ml was added. The cells were incubated for 45 minutes in the presence or absence of test compounds at 1nM-10uM dose ranges (compounds were solubilized in Dimethyl- sulfoxide/Ethanol such that the final solvent concentration in the culture medium was 0.5% Dimethyl sulfoxide/0.5% Ethanol). Bacterial lipopolysaccharide (E. coli 055:B5 [LPS] from Sigma Chemicals Co.) was then added at 100 ng/ml in 10 ml Phosphate Buffered Saline (PBS) and cultures incubated for 16-18 hours at 37°C in a 5% CO_2 incubator. At the end of the incubation period, culture supernatants were removed from the cells, centrifuged at 3000 revolutions per minute (rpm) to remove cell debris and .05 ml of the supernatant assayed for TNF activity using the radioimmunoassay described below.

Section II: Radioimmunoassay procedure for TNF activity

The assay buffer consisted of 0.01M $NaPO_4$, 0.15M NaCl, 0.025M EDTA and 0.1% sodium azide at pH 7.4. Human recombinant TNF (rhTNF) obtained using the procedure of Chen et al., Nature, 330:581-583 (1987) was iodinated by a modified Chloramine-T method described in Section III below. To samples (50 µl culture supernatants) or rhTNF standards, a 1/9000 dilution of polyclonal rabbit anti-rhTNF (Genzyme, Boston, MA) and 8000 cpm of ^{125}I-TNF was added in a final volume of 400 µl buffer and incubated overnight (18 hours) at 4°C. Normal rabbit serum and goat anti-rabbit IgG (Calbiochem) were titrated against each other for maximum precipitation of the anti-rhTNF. The appropriate dilutions of carrier normal rabbit serum (1/200), goat anti-rabbit IgG (1/4) and 25 Units heparin (Calbiochem) were allowed to precipitate and 200 µl of this complex was added per assay tube and incubated overnight at 4°C. Tubes were

centrifuged for 30 minutes at 2000 rpm, supernatants were carefully aspirated, and radioactivity associated with the pellets measured in a Beckman Gamma 5500 counter. The logit-log linear transformation curve was used for the calculations. The concentrations of TNF in the samples was read from a standard curve of rhTNF that was linear in the 157 to 20,000 pg/ml range.

Section III: Radioiodination of rhTNF

Iodination of rhTNF was performed using a modified chloramine-T method of Frolik et al., J. Biol. Chem., 259:10995-11000 (1984). Briefly, 5 mg of rhTNF in 5 ml of 20MM Tris ph 7.5, was diluted with 15 ml of 0.5M KPO_4 and 10 ml of carrier free ^{125}I(100mCi/ml;ICN). To initiate the reaction, a 5ml aliquot of a 100mg/ml (aqueous) chloramine-T solution was added. After 2 minutes at room temperature, an additional 5 ml aliquot was added followed 1.5 minutes later by a final 5 ml addition of chloramine-T. The reaction was stopped 1 minute later by sequential addition of 20 ml of 50mM Sodium Metabisulfite, 100 ml of 120mM Potassium Iodide and 200 ml of 1.2 mg/ml Urea. The contents were mixed and the reaction mixture was passed over a pre-packed Sephadex G-25 column (PD 10 Pharmacia), equilibrated and eluted with Phosphate Buffered Saline pH 7.4 containing 0.25% gelatin. The peak radioactivity containing fractions were pooled and stored at -20°C. Specific activity of ^{125}I-TNF was 80-100 mCi/mg protein. Biological activity of iodinated TNF was measured by the L929 cytotoxicity assay of Neale, M.L. et al., Eur. J. Can. Clin. Oncol., 25(1):133-137 (1989) and was found to be 80% that of unlabeled TNF.

Section IV: Measurement of TNF- ELISA:

Levels of TNF were also measured using a modification of the basic sandwich ELISA assay method described in Winston et al., Current Protocols in Molecular Biology, Page 11.2.1, Ausubel et al., Ed. (1987) John Wiley and Sons, New York, USA The ELISA employed a murine monoclonal anti-human TNF antibody, described below, as the capture antibody and a polyclonal rabbit anti-human TNF , described below, as the second antibody. For detection, a peroxidase-conjugated goat anti-rabbit antibody (Boehringer Mannheim, Indianopolis, Indiana, USA, Catalog # 605222) was added followed by a substrate for peroxidase (1mg/ml orthophenylenediamine with 0.1% urea peroxide). TNF levels in samples were calculated from a standard curve generated with recombinant human TNF produced in E. Coli (obtained from SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA).

Section V: Production of anti-human TNF antibodies:

Monoclonal antibodies to human TNF were prepared from spleens of BALB/c mice immunized with recombinant human TNF using a modification of the method of

Kohler and Millstein, Nature 256:495 (1975), the entire disclosure of which is hereby incorporated by reference. Polyclonal rabbit anti-human TNF antibodies were prepared by repeated immunization of New Zealand White (NZW) rabbits with recombinant human TNF emulsified in complete Freund's adjuvant (DIFCO, IL., USA).

Results:

It has been determined that 1,3-di-cyclopropylmethyl-8-amino xanthine demonstrated an IC_{50} of about 0.05 μM in the in-vitro TNF production assay system.

UTILITY EXAMPLE B

Endotoxin Shock in D-gal-Sensitized Mice

The protocol used to test the compound of the method of the subject invention was essentially as has been described in Galanos et al., Proc. Nat'l Acad. Sci USA, 76:5939-43 (1979) whose disclosure is herein incorporated by reference. Briefly, D-gal (D(+) Galactosidase) sensitizes various strains of mice to the lethal effects of endotoxin. The administration of D-gal (300-500mg/kg) intra-venously (i.v.) sensitizes the mice to doses of lipopolysaccharide (LPS) as low as 0.1μg. Briefly, male C57BL/6 mice, obtained from Charles River Laboratories (Stone Ridge, New York, USA) of 6-12 weeks of age were injected i.v. with 0.1 μg of LPS from Salmonella typhosa (Difco Laboratories, Detroit, Michigan, USA) admixed with D(+)-gal (Sigma; 500 mg/kg) in 0.20-0.25 ml pyrogen-free saline. Compounds to be tested were administered at various times prior to or following the i.v. injection of LPS/D-gal. In this model, the control animals usually die 5-6 hr. following the injection of LPS, although on occasion deaths are seen between 24 and 48 hr.

Measurement of TNF Activity

Plasma levels of TNF were measured using a modification of the basic sandwich ELISA method described in Winston et al., Current Protocols in Molecular Biology, Pg. 11.2.1, Ausubel et al., Ed. (1987) John Wiley and Sons, New York , USA. The Elisa employed a hampster monoclonal anti-mouse TNF (Genzyme, Boston, MA, USA) as the capture antibody and a polyclonal rabbit anti-murine TNF (Genzyme, Boston, MA, USA) as the detecting antibody. TNF levels in mouse samples were calculated from a standard curve generated with recombinant murine TNF (Genzyme, Boston, MA , USA). TNF levels determined by ELISA correlated with levels detected by the L929 bioassay of Ruff et. al., J. Immunol. 125:1671-1677 (1980), with 1 Unit of activity in the bioassay corresponding to 70 picograms (pg) of TNF in the ELISA. The ELISA detected levels of TNF down to 25 pg/ml.

Results:

It has been determined that 1,3-di-cyclopropylmethyl-8-amino xanthine also demonstrated a positive in-vivo response in the above noted utility model, having an ED_{50}

for reduction of serum TNF of about 0.1mg/kg intraperitoneally. The compound demonstrates a 100% survival of the animals at this dose.

UTILITY EXAMPLE C

HIV in vitro monocyte assay

The effects of the compounds of Formula (I) on the in vitro inhibition of HIV production of chronically infected cells is examined using the following protocol.

Isolation of HIV-infected cell lines.

Clonal HIV-infected cell lines were derived by infecting a culture of the H9 T-cell line with the $HTLV_{IIIB}$ strain of human immunodeficiency virus (HIV_{IIIB}) and culturing the cells for 5 weeks during which a chronically-infected cell line developed. Clones were derived from this culture by limiting dilution plating in a 1:1 mixture of RPMI 1640 + 15% fetal bovine serum and H9 cell-conditioned medium. Clones were expanded to approximately 4×10^7 cells, aliquots were frozen and subsequent cultures were assayed for their production of HIV with and without stimulation by TNF or other recombinant cytokines as described below.

Induction of HIV.

HIV induction was assayed by culturing clonal HIV- infected cell lines for four days in the presence of the material to be tested for inducing activity. For measurement of inhibition of HIV induction, cultured human monocytes were stimulated to produce cytokines by treatment with lipopolysaccharide (LPS) in the presence or absence of test compounds for 18 hours. At the end of stimulation, supernatant medium from the monocyte cultures was collected, frozen in aliquots at -80° C, and the concentrations of TNF, IL1-ß, and IL-6 were determined in one aliquot by ELISA. The monocyte supernatants were then diluted into complete RPMI growth medium to provide an optimally inducing concentration of TNF in the case of the positive control (LPS stimulated-) monocyte sample. Optimal induction was achieved at 10 to 100 units of TNF /ml (0.5 to 5.0 ng/ml), depending upon the indicator cell line. Supernatants from experimentally-treated monocyte cultures were diluted by the same factor as that used for the positive control in each experiment.

Following the four day experiment, culture supernatant fluid (90 µl) was removed from the HIV-infected cell line and added to 5% (v/v) Triton-X-100 (10 µl; Sigma Chemical Company) to liberate reverse transcriptase from HIV particles and inactivate the virus. Eight cultures were evaluated for each treatment in two or more experiments. Samples were stored at -80° C until assayed for reverse transcriptase activity.

Reverse transcriptase assay.

HIV reverse transcriptase was assayed by a modified version of the microtiter assay of Goff et al. (J. Virol. 38:239- 248,1981). Incorporation of ^{32}P-dTTP into polynucleotide on an oligo- A:poly-dT template:primer was measured by filtering the reaction products with an NA45 membrane filter (Schleicher and Scheull) on a dot-blot apparatus, and either autoradiography or AMBIS quantitation or both. Duplicate reverse transcriptase assays were performed on all samples.

Statistical methods.

Statistical significance of results was calculated using the COMPARE functions of the RS/Explore software package.

RESULTS:

Most clonal HIV-infected cell lines express increased levels of HIV in response to TNF in the culture medium. Ten clonal H9 cell lines were cultured in quadruplicate for four days with or without 5 ng of rTNF /ml of RPMI 1640 + 10% fbs. At the end of the experiment, reverse transcriptase in the culture medium was measured to determine the level of HIV production by each culture. Eight out of the ten cell lines tested produced increased levels of HIV when cultured in the presence of TNF. A particular clone, cell line 3.7 responded to TNF in a typical fashion, known to one skilled in the art. The cell line 3.7 was chosen an used to evaluate induction in response to monocyte supernatants.

Clonal HIV-infected cells express increased levels of HIV not only in response to TNF but also to supernatant fluid from LPS-stimulated, but not control, cultured human monocytes. Cell line 3.7 was cultured for four days in medium supplemented with either recombinant TNF-α at 5ng/ml, fluid from cultured human monocytes or fluid from cultured human monocytes that were stimulated with LPS, and the levels of HIV produced were measured four days later by determining reverse transcriptase levels in the supernatant fluid.

Cell line 3.7 was chosen because it reproducibly induced HIV in response to recombinant TNF. The results here were also obtained with cell lines 4,7,3,U-1 and ACH-2. Commercially available cell lines are also useful in this assay.

Supernatants from cultured human monocytes stimulated with LPS in the presence of inhibitors of TNF synthesis have a reduced HIV-activating activity compared to supernatants from monocytes stimulated with LPS in the absence of inhibitors. HIV-infected clonal cell line 3.7 was cultured for four days in medium supplemented with supernatants from control human monocytes, human monocytes stimulated with LPS, and human monocytes stimulated with LPS in the presence of 10μM a compound of Formula (I), a 1,3-di-cyclopropylmethyl-8-amino xanthine.

This assay demonstrates that the compounds of Formula (I), as inhibitors of TNF will inhibit HIV induction by LPS-stimulated monocyte supernatants if present

during LPS- stimulation. In particular, 1,3-di-cyclopropylmethyl-8-amino xanthine exhibited an HIV Inhibition of (%+/-Error)+/- (75-5) at a concentration of 10 μM.

The percent inhibition in parentheses comes from a representative experiment. Actual inhibition in any experiment may vary depending upon the monocyte donor, dose response curve of the test HIV- infected T cell line, and dilution of monocyte supernatant.

UTILITY EXAMPLE D

In vivo inhibition of influenza virus-induced TNF

The effects of the compounds of Formula (I) on virus induced TNF production in vivo was examined using the following protocol.

Mice:

Age-matched, female specific pathogen free (Balb/c x C57B/6) F_1 ($CB6F_1$) mice were purchased from Charles River Laboratories. Mice were 4-10 weeks old at arrival. Mice used for LD_{50} determinations were between 8-14 weeks old.

Virus Production:

The type A influenza virus strain A/PR/8/34 (H1N1 subtype) was propagated in the allantoic cavity of 10 day old fertilized eggs. After incubating eggs for 48 hours, they were refrigerated for at least 2-1/2 hours before harvesting allantoic fluid. Pooled allantoic fluid was centrifuged (2000 rpm, 15 min, 4°C) to remove cells, and then divided into aliquots for storage at -70°C.

In Vitro Virus Titration:

Virus was quantitated in an in vitro microassay using Madin-Darby canine kidney (MDCK) cells to establish the 50% tissue culture infectious dose ($TCID_{50}$). Serial dilutions of virus or lung homogenate (in medium plus 2.5 ug/ml trypsin) was added (in quadruplicate) to round-bottomed microtiter wells containing adherent MDCK cells. After 5 days incubation at 37°C (6% CO_2), 50 ul of 0.5% chick red blood cells were added per well, and agglutination was read after 1 hour at room temperature. The $TCID_{50}$ dose was calculated using the SAS version 5 program for 50% effective dose (ED_{50}) estimation for a Binary Dose-Response Assay, (SAS/Statuser's Guide, Vol. 2, SAS Institute, Cary, NC (1985) and "Applied Categorical Data Analysis", Marcal Dekker Inc., Publishers, N.Y., N.Y.).

In Vivo Virus Challenge:

Freshly thawed virus was serially diluted in tenfold steps (10^{-1}-10^{-8}) in sterile PBS with .05-1% bovine serum albumin; dilutions were kept on ice until use. $CB6F_1$ mice were anesthesized by brief exposure to methoxyfluorane (metofane; Pittman

Moore Co.) soaked paper towels and were challenged intranasally with 50 ul virus. A dose equivalent to 2 LD_{50} was used in these experiments.

Collection of Samples from Virus Challenged Mice:

Sera: Mice were bled from the orbital venous plexus using a heparinized pasteur pipet. Blood from 3-4 mice was pooled and centrifuged at 15K for 15 minutes; plasma was divided into aliquots and frozen at $^{-}20^{o}C$.

Lung Homogenates: Lungs from mice that were infected intranasally 3 days earlier were removed aseptically and placed in vials (1 lung per vial) containing 1 micron glass beads (Biospec Products, Bartlesville, OK) (about 1/4 full) and 1 ml Eagles minimal essential medium with penicillin and streptomycin. The lungs were homogenized for 1 minute using a mini-bead beater (Biospec Products); the vials were then centrifuged at 3000 rpm for 15 min at $4^{o}C$, and the lung supernatants were frozen at $^{-}20^{o}C$.

Bronchoalveolar Washes: Mice were euthanized by cervical dislocation and wet down with alcohol. Spleen was removed to expose diaphragm. The diaphragm was cut to collapse lungs, and rib cage cut away to expose trachea. The trachea was snipped about 3-5 mm above the lungs and 1 ml PBS was injected into the lungs through a blunt-end 19 gauge needle. The fluid was recovered into the syringe (~ 60-80% starting volume), spun at 2000 rpm for 15 min. to remove cells and debris. Aliquots of supernatants were frozen at $^{-}20^{o}$ prior to assay.

Dosing with a compound of Formula (I): A compound of Formula (I), a 1,3-di-cyclopropylmethyl-8-amino xanthine (hereinafter referred to as compound (1)) was initially dissolved in DMSO/EtOH and brought up to volume with FBS/saline to equal a final concentration of 1 mg/ml in 5% DMSO, 5% Etoh, 40% FBS and 50% normal saline. Injections were administered ip (0.2 ml per mouse) to equal 0.2 mgs per 20 gms mouse (10 mg/kg); or other doses as noted below.

TNF Elisa Assay: The TNF Elisa assay is the same as that described in Utility Example B above.

Rationale and Overall objective: There is a lack of an HIV animal model for testing the activity of inhibitors of the HIV virus, and further one which could be readily monitored in vivo. Initial reports in the literature demonstrate that influenza-infected monocytes produce TNF and therefore lead to the choice of the influenza model as one which is useful for in vivo monitoring of the compounds of Formula (I) as inhibitors of TNF.

Mouse influenza model: Brief Description. Human influenza viruses replicate in mouse lungs, but do not cause overt disease. However, pathogenicity for mice can be increased by serial passage in mouse lungs. The mouse-adapted viruses cause a lethal pneumonia, and not an upper respiratory tract infection as in uncomplicated human

influenza. In murine influenza, virus replication is restricted to the lung, and is accompanied by a massive inflammatory cell infiltrate. It is well documented that pulmonary interferon levels rise during murine influenza (Wyde, et. al) and a recent report documented non-quantitative increases (by bioassay) in both IL-1 and TNF levels in bronchoalveolar washes of influenza infected mice (Vacheron, et al).

RESULTS:

In vivo TNF production.

In an initial study, blood and bronchoalveolar wash (BAW) samples were obtained from mice infected intranasally with A/PR/8/34 virus (2 LD_{50}) at .5, 1, and 6 hours, and on days 1, 3, 5, 7, 9 and 14 post-infection. Analysis of pooled samples (3 mice per group) by TNF Elisa assay confirmed that TNF was produced in the lung, but no TNF was detected in the blood at any timepoint. In a follow-up experiment, BAW were assayed individually (n=5 mice per group), and lung homogenates were also prepared for TNF analysis and virus titrations. The results demonstrate that TNF levels were increased on days 2 through 7 post-infection, whereas virus replication was evident by 24 hours. Although virus titers began to decline after day 3, TNF levels remained elevated. The results suggest that virus replication triggered events (ie-inflammatory cell infiltrate) leading to local TNF production, and that pulmonary TNF levels were sustained as the virus was being cleared from the lung.

Effect of Compound (1) on Influenza induced TNF Production.

A treatment protocol was designed based on the kinetics of TNF production seen in the lung. Treatment was initiated on day 1, which preceded any detectable rise in lung TNF. Mice were administered compound (1) at 10 mg/kg ip daily, and BAW performed 2 hours after the last injection. Samples were taken on days 2 or 3 post-infection. TNF levels in BAW were significantly reduced on both days 2 and 3 in comparison to untreated controls also, with a maximal reduction of 67% (n=3 of 3 experiments). TNF levels were significantly reduced in lung homogenates on day 3 only (n=1 of 1 experiments) (not shown). A dose titration of compound (1) demonstrated that the compound was active (~ 50% reduction of BAW TNF) at 1 mg/kg, and no effect was seen at 0.1 mg/kg.

CONCLUSIONS:

These studies demonstrate that therapeutic administration of the compounds of Formula (I) can reduce virus-induced TNF production in vivo. The data also suggest that the compound will be effective at reducing TNF levels in tissue as well as in the circulation.

Effect of Compound (1) on Influenza Virus Titers in Mouse Lung.

Virus titers were significantly reduced on days 2 through 5 in mice treated with 1-10 mg/kg compound (1) on days 1,2 and 3 post infection .

Conclusion: These data provide evidence that treatment with the compounds of Formula (I) reduces lung virus titers, and thus may be directly beneficial in human influenza infection.

Effect of compound (1) on Survival in a Lethal Challenge Model of Murine Influenza.

In mice given a lethal intranasal challenge dose of A/PR/8/34 influenza virus, there was a significant improvement in survival in mice treated with 10 mg/kg compound (1) daily, but survival in groups of mice treated with lower doses, or treated with 0.1 -10 mg/kg during days 1, 2, and 3 post infection, was not significantly different than vehicle treated controls.

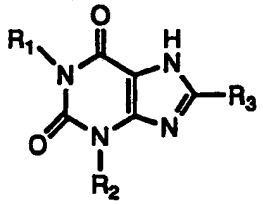
Conclusion: Treatment of influenza challenged mice with compound (1) at doses shown to reduce lung TNF levels resulted in a moderate improvement in survival which was significant when mice were dosed daily with 10 mg/kg. Thus, the reduction of TNF was not detrimental in this infection. Although the effects on survival were not dramatic, it is possible that more significant benefit might be demonstrated by measuring earlier endpoints such as clinical symptoms, but this is not readily done in the mouse model, where the infection is confined to the lower respiratory tract. Treatment with the compounds of Formula (I) therefore suggest reduced morbidity and/or mortality in human influenza, where the levels of nasal virus shedding generally correlate with clinical symptoms.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the are can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

CLAIMS:

What is Claimed is

1. A method of treating yeast or fungal infections in a mammal in need thereof which comprises administering to such mammal an effective, TNF production inhibiting amount of a compound of Formula (I):



(I)

wherein

R_1 and R_2 each independently represent alkyl or $-(CH_2)_m-A$; provided that at least one of R_1 and R_2 represent $-(CH_2)_m-A$;

m represents zero or an integer 1, 2 or 3;

A represents a substituted or unsubstituted cyclic hydrocarbon radical;

R_3 represents a halogen atom, a nitro group, or a group $-NR_4R_5$;

R_4 and R_5 each independently represent hydrogen, alkyl or alkylcarbonyl; or R_4 and R_5 together with the nitrogen to which they are attached form an optionally substituted heterocyclic group; and the pharmaceutically acceptable salts thereof.

2. The method according to claim 1, wherein R_1 represents $-(CH_2)_m-A$.

3. The method according to claim 1, wherein R_1 and R_2 both independently represent $-(CH_2)_m-A$.

4. The method according to claim 3 wherein A represents a substituted or unsubstituted C_{3-8} cycloalkyl group.

5. The method according to claim 4, wherein m represents 1.

6. The method according to claim 5, wherein A represents a substituted or unsubstituted cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl group.

7. The method according to claim 6, wherein A represents a cyclopropyl group or a cyclobutyl group.

8. The method according to claim 7, wherein R_3 is nitro, or $-NR_4R_5$ wherein R_4 is hydrogen and R_5 is hydrogen or alkylcarbonyl.

9. The method according to claim 8, wherein R_4 or R_5 is hydrogen.

10. The method according to claim 9, wherein A represents a cyclopropyl group.

11. The method according to claim 3, wherein the $-NR_4R_5$ term of R_3 represents a saturated heterocyclic group having single or fused rings , and the saturated heterocyclic ring is a single ring of 5 to 7 atoms which ring optionally comprises up to two additional heteroatoms selected from O, N or S.

12. The method according to claim 1 wherein the compound is selected from the group consisting of:
1,3-di-n-butyl-8-nitro xanthine;
1,3-di-cyclopropylmethyl-8-nitro xanthine;
1,3-di-cyclobutylmethyl-8-nitro xanthine;
1,3-di-cyclopentylmethyl-8-nitro xanthine;
1,3-di-cyclohexylmethyl-8-nitro xanthine;
1,3-di-n-butyl-8-amino xanthine;
1,3-di-cyclopropylmethyl-8-amino xanthine;
1,3-di-cyclobutylmethyl-8-amino xanthine;
1,3-di-cyclopentylmethyl-8-amino xanthine;
1,3-di-cyclohexylmethyl-8-amino xanthine;
1,3-di-cyclopropyl-8-amino xanthine;
1,3-di-n-butyl-8-acetamido xanthine;
1,3-di-n-butyl-8-chloro xanthine;
1,3-di-n-butyl-8-bromo xanthine;
1,3-di-cyclopropylmethyl-8-chloro xanthine;
1,3-di-cyclohexyl-8-chloro xanthine;
1,3-di-n-butyl-8-piperidino xanthine;
1,3-di-cyclopropylmethyl-8-morpholino xanthine;
1,3-di-n-butyl-8-pyrrolidinyl xanthine;
1,3-di-cyclopropylmethyl-8-pyrrolidinyl xanthine;
1,3-di-cyclopropylmethyl-8-piperidinyl xanthine;
1,3-di-cyclohexylmethyl-8-piperidinyl xanthine;
1,3-di-cyclohexylmethyl-8-bromo xanthine; and
1,3-di-cyclohexyl-8-nitro xanthine; or if appropriate, a pharmaceutically acceptable salt thereof.

13. The method according to Claim 1 wherein the TNF inhibiting compound is 1,3-di-cyclopropylmethyl-8-amino xanthine or a pharmaceutically acceptable salt thereof.

14. The method of Claim 1 wherein the compound is administered orally, parenterally, topically or by inhalation.

15. The method according to Claim 1 wherein the fungal infection is fungal meningitis.

16. The method according to Claim 1 wherein the fungus is Candida.

17. A method of treating fungal infections in a mammal in need thereof by co-administering an effective amount of a second anti-fungal agent, and an effective amount of a compound of Formula (I) according to Claim 1, or a pharmaceutically acceptable salt thereof .

18. A method of treating fungal infections in a mammal in need thereof by co-administering an effective amount of an anti-fungal agent, and an effective amount of 1,3-di-cyclopropylmethyl-8-amino xanthine or a pharmaceutically acceptable salt thereof.

19. The method according to Claim 18 wherein the fungal infection is fungal meningitis.

20. The method according to Claim 18 wherein the fungus is Candida.

21. The method according to Claim 18 wherein the second anti-fungal agent is Amphotericin B.

22. A method for inhibiting or reducing the toxicity of anti-fungal, anti-bacterial, or anti-viral agents by administering an effective amount of 1,3-di-cyclopropylmethyl-8-amino xanthine or a pharmaceutically acceptable salt, to a mammal in need thereof who is also administered an antifungal, anti-bacterial or anti-viral agent.

23. The method according to Claim 22 wherein the anti-fungal agent is Amphotericin B.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01496

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/52
US CL :514/263, 265

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/263, 265, 886, 958

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A, 0 389 282 (BEECHAM-WUELFING GMBH & CO.) 26 September 1990. See page 5 (lines 47-53), page 6 (line 37) to page 7 (line 5), page 9 (line 36) to page 10 (line 17) and the Abstract.	1-33
Y	Journal of Cell Biology, Volume 107, October 1988, (B. SHERRY), "Cachectin/Tumor Necrosis Factor Exerts Endocrine, Paracrine, and Autocrine Control of Inflammatory Responses". See page 1269 and pages 1271 to 1274.	1-33
Y	Proceedings of the National Academy of Science, U.S.A., Volume 87, January 1990, (POLI), "Tumor necrosis factor functions in an autocrine manner in the induction of human immunodeficiency virus expression", pages 782-785.	16,17,19-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be part of particular relevance	
"E" earlier document published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search 23 APRIL 1993	Date of mailing of the international search report 10 MAY 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer NATHAN M. NUTTER Telephone No. (703) 308-2351

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01496

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Endocrinology, Volume 124, No. 3, 1989, (R.A. JOHNSON), "Tumors Producing Human Tumor Necrosis Factor Induce Hypercalcemia and Osteoclastic Bone Resorption in Nude Mice", pages 1424-1427.	18